Comparison of the Chemical Composition and Bacterial Population Structure of Dairy Waste Before and After Aerobic and Anaerobic Digestion

Jeffery A. McGarvey[1]*, William G. Miller[1], Jeremy R. Lathrop[1], Ruihong Zhang[2], Frank Mitloehner[3], and Yangua Ma[2]
Agricultural Research Service, U. S. Department of Agriculture, Albany CA[1].
The Department of Biological and Agricultural Engineering[2] and Animal Science[3], The University of California, Davis, CA.

Introduction: California is the largest dairy producing state in the United States, housing over 2.5 million dairy cows on approximately 2,300 dairies, with the average farm maintaining 1,000 cows. The average 450 kg dairy cow produces approximately 37 kg of waste (manure and urine) per day, thus a 1,000 cow dairy produces 37,000 kg of waste per day or 13.5 million kg of waste per year. The waste is usually held in storage lagoons until it can be applied to agricultural fields as a soil amendment / fertilizer for crops destined for animal or human consumption. The average herd size in California has increased by approximately 8% a year for the last ten years and new problems associated with the waste stream have emerged. For example, many of the larger dairies produce more waste than they can apply to nearby fields due to excessive nutrient levels (e.g. nitrogen, phosphate, potassium etc.) and transporting waste to distant agricultural fields is an economic liability. Cow manure has also been associated with pathogenic bacteria such as E. coli O157:H7, Salmonella sp., Campylobacter sp., Mycobacterium avium subsp. paratuberculosis, and crops fertilized with this material may transmit these pathogens to the consumer. One possible solution to these problems is to treat the waste before it enters the storage lagoons. The most commonly used treatment methodologies for both municipal and agricultural wastes are aerobic and anaerobic digestion. Previous studies have shown these techniques are effective for nutrient and pathogen reduction but little is known about the microbial population dynamics associated with these processes.
Because cultivation methods are estimated to support the growth of less than 1% of the naturally occurring biodiversity, the use of 16S rDNA analysis has proven to be a powerful tool to describe the microbial population structure of the human gut and soil, and to compare the populations associated with different types of dairy waste storage lagoons. In this study, we used 16S rDNA sequence analysis to compare the bacterial population dynamics in dairy waste treated by aerobic or anaerobic digestion followed by storage in simulated waste storage lagoons, to the dynamics in untreated waste. This was accomplished by pumping fresh dairy waste through lab scale aerobic and anaerobic digesters and holding the effluent in stagnant storage tanks that simulated a dairy waste storage lagoon or simply holding the waste in simulated storage lagoons. Samples were collected from the waste material, the digesters and the storage tanks for a period of 6 months and monitored for their chemical composition and bacterial population structure. Our results confirm that both aerobic and anaerobic digestion are more effective at reducing nutrient levels as compared to storage alone and that each treatment method has a unique effect on the bacterial population structure in waste.

Methods and Results: Fresh dairy cow waste (manure and urine < 12 h post excretion) was diluted to 4 % total solids (TS) with tap water, loaded into a feed tank maintained at 4oC, and used to feed the aerobic and anaerobic reactors described schematically in Figure 1. Aerobic digestion was performed at room temperature (approximately 25 oC) in a 2 L vessel with a hydraulic retention time (HRT) of 5 days. Atmospheric air was pumped continuously through the vessel to maintain a dissolved oxygen concentration of approximately 2 ppm. Effluent from the aerobic digester was collected and held in a 100 L storage vessel for the duration of the experiment. Anaerobic digestion was performed in a 4 L vessel maintained at 35oC. The contents of the anaerobic digester was mixed twice daily by re-circulating the headspace gas through the liquid for 2 min. The anaerobic digester had a hydraulic retention time of 20 days, and the effluent was collected and stored in a 100 L tank for the duration of the experiment. In addition to the vessels described above, feed material was pumped into untreated control tanks at the same rate as the aerobic and anaerobic digesters. This material received no mixing and was maintained at room temperature for the duration of the experiment. A sampling scheme was developed such that the feed material was assayed weekly, the aerobic and anaerobic digesters were sampled biweekly, and the digester effluent storage tanks and the control tanks were sampled monthly.

Conclusion and Significance: Both aerobic and anaerobic treatment followed by storage were superior to storage alone for the reduction of the total solids, BOD, phosphate and coliform bacteria. In addition to these reductions, each system had unique remediation properties. For example, aerobic treatment significantly reduced both total nitrogen and ammonia levels. These reductions are likely the result of the deamination of proteins and peptides and the hydrolysis of urea to ammonia by ruminant bacteria. In the oxygen rich environment of the aerobic digester, ammonia became nitrified by ammonia oxidizing bacteria of the genus Nitrosomonas, which were only observed in the aerobic treatment system (data not shown). When the oxidized nitrogen species entered the anoxic conditions of the storage tank they were denitrified to volatile nitrogen containing gasses that escaped into the atmosphere. In addition, some ammonia was likely volatized and assimilated by the bacteria. In the anaerobic system significant reductions in sulfate and total sulfur were observed. This loss is likely explained by dissimulatory sulfate reduction to form hydrogen sulfide and other volatile sulfur containing compounds, and to a lesser extent by assimilation.
At the phylum level, the feed material derived 16S rDNA library was very similar to a library constructed from dairy waste reported previously. In both of these libraries the greatest percentage of sequences were members of the phylum Firmicutes (74% in this study vs. 77% in the previous), followed by the phyla Bacteroidetes (16% vs. 7%), Actinobacteria (11% vs. 9%) and Proteobacteria (3% vs. 5%). The feed material library also possessed similarities to libraries derived from human feces (11), the gastrointestinal tract of pigs (23) and to a lesser extent broiler chicken litter. The aerobic digester effluent library had similarities to a library derived from a circulated dairy waste lagoon. In these libraries the phylum Proteobacteria was most prominent followed by the Firmicutes, Bacteroidetes and Actinobacteria. However, these libraries differed in the abundance of the phylum Firmicutes which represented 26.8% of the circulated waste lagoon derived library, as compared to only 9.5% in the aerobic digester effluent derived library. This difference may be explained by the growth inhibition of many of the obligate anaerobic members of the Firmicutes in the aerobic digester which maintained an oxygen concentration of 2 ppm as compared to the circulated waste lagoon which was essentially anoxic. The predominance of Firmicutes 16S rDNA sequences increased to 21.7%, after storage in a simulated waste lagoon, making it more closely resemble the library derived from the circulated dairy waste lagoon reported previously. The increased number of Firmicutes-like sequences may be explained by the anoxic conditions encountered in the simulated waste lagoon that support the growth of the obligate anaerobic species within this phylum. The library generated from the anaerobic digester was similar to a library derived from a stagnant dairy waste lagoon; however, the relative levels of the Proteobacteria and Bacteroidetes were inverted. Subsequent storage of the anaerobic digester effluent did little to change the bacterial community structure at the phyla level, with only a slight increase in the phyla Deinococcus-Thermus observed.
Of the ten most prevalent OTUs in the waste derived library, most have been recovered previously in dairy waste (Feed 7), wastewater lagoons (Feed 2, 5, 6, and 8) or the gastrointestinal tract of swine (Feed 3). Storage without treatment does little to change the predominance of these OTUs, with the vast majority resembling those isolated previously in dairy waste (Cont 1), dairy wastewater (Cont 2, 4, 6, 7 and 9) or swine waste (Cont 3 and 10). Aerobic digestion and subsequent effluent storage resulted in the greatest changes in the most commonly observed OTUs, with only 3 of 20 OTUs previously associated with waste (Aero3, 6 and AS7) and the rest were similar to environmental isolates. Anaerobic treatment and subsequent storage resulted in fewer changes in the OTUs identified. Many of the ten most prevalent OTUs have been recovered previously in manure or stagnant dairy waste lagoons (Anaero 4, 6, 8, 9, and 10 and ANS1, 3, 6, 8, and 9).

Figure 1. Schematic Diagram of Digester Configuration

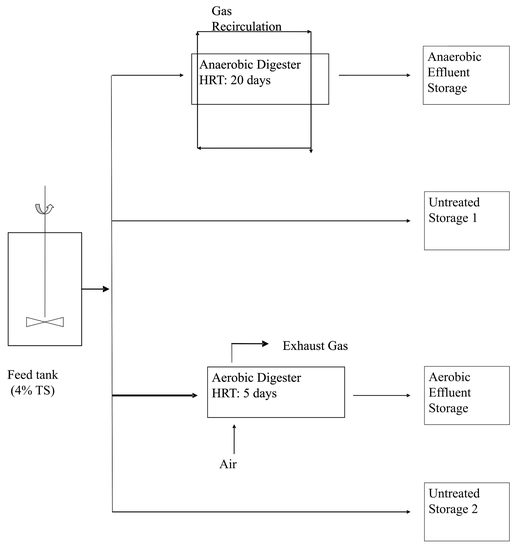

Figure 2 Bacterial Population Dynamics of Aerobically and Anaerobically Treated Dairy Waste

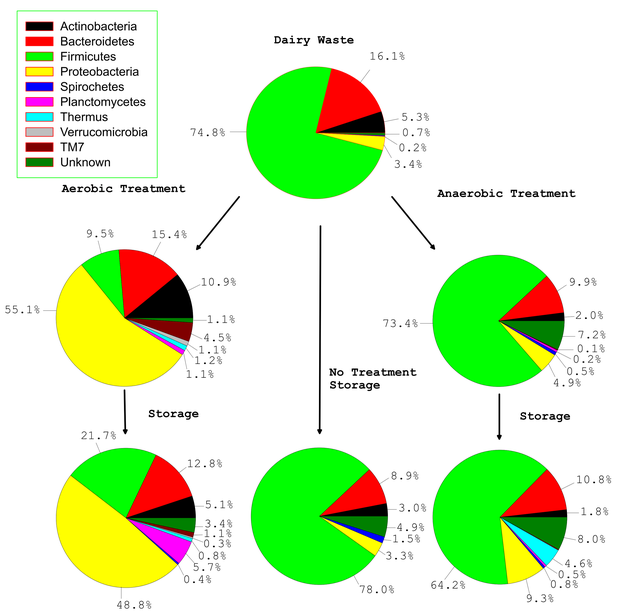

Table 1. Chemical and cultural analysis of aerobic and anaerobic waste treatment systems

A. Aerobic Treatment

Parameter	Feed Material Average (Range)	Digester Effluent Average (Range)	Effluent Storage Tank Average (Range)	Untreated Control Average (Range)
Total Solids	44,000 (29-58,000)	30,000 (16-45,000)	14,000 (7-29,000)	28,000 (17-54,000)
BOD	14,900 (13-18,000)	3,500 (2-4,500)	1,500 (870-2,200)	10,600 (8-15,000)
Total N	2,900 (13-18,000)	2,200 (1-5,000)	1,500 (600-4,300)	3,000 (1-6,000)
NH_4	750 (400-1,600)	100 (60-180)	230 (100-500)	1,100 (900-1,500)
S	330 (100-600)	360 (200-600)	200 (100-500)	230 (200-400)
SO_4	459 (200-950)	518 (250-950)	204 (90-280)	140 (100-200)
P_2O_5	720 (200-900)	670 (200-900)	300 (200-500)	610 (500-1,100)
K,O	2,200 (1-3,000)	2,200 (1-3,200)	2,000 (1,000-3,200)	1,900 (1,200-3,000)
Na	570 (300-1,000)	600 (400-900)	630 (500-800)	540 (500-600)
APC	$5.4x10^7$ ($0.04-7.2x10^7$)	$6.3x10^7$ ($0.02-2.8x10^8$)	$9.1x10^7$ ($0.08-2.7x10^7$)	$3.0x10^8$ ($0.5-5.0x10^8$)
AnPC	$1.7x10^7$ ($0.1-5.1x10^7$)	$1.4x10^8$ ($0.01-4.4x10^8$)	$1.7x10^8$ ($0.02-8.6x10^8$)	$1.5x10^8$ ($0.04-5.1x10^8$)
CPC	$1.8x10^7$ ($0.02-1.0x10^8$)	$9.9x10^7$ ($0.0-1.7x10^8$)	$1.4x10^8$ ($0.0-6.1x10^8$)	$7.2x10^8$ ($0.03-2.9x10^9$)

B. Anaerobic Treatment

Parameter	Feed Material Average (Range)	Digester Effluent Average (Range)	Effluent Storage Tank Average (Range)	Untreated Control Average (Range)
Total Solids	44,000 (29-58,000)	25,000 (16-36,000)	18,000 (7-29,000)	28,000 (17-54,000)
BOD	14,900 (13-18,000)	1,900 (2-4,500)	2,200 (870-2,200)	10,600 (8-15,000)
Total N	2,900 (13-18,000)	2,600 (1-5,000)	2,400 (600-4,300)	3,000 (1-6,000)
NH_4	750 (400-1,600)	1,000 (60-180)	1,000 (100-500)	1,100 (900-1,500)
S	330 (100-600)	140 (200-600)	130 (100-500)	230 (200-400)
SO_4	459 (200-950)	768 (250-950)	160 (90-280)	140 (100-200)
P_2O_5	720 (200-900)	500 (200-900)	250 (200-500)	610 (500-1,100)
K,O	2,200 (1-3,000)	1,800 (1-3,200)	1,500 (1,000-3,200)	1,900 (1,200-3,000)
Na	570 (300-1,000)	500 (400-900)	510 (500-800)	540 (500-600)
APC	$5.4x10^7$ ($0.04-7.2x10^7$)	$2.2x10^7$ ($0.02-2.8x10^8$)	$2.7x10^7$ ($0.08-2.7x10^7$)	$3.0x10^8$ ($0.5-5.0x10^8$)
AnPC	$1.7x10^7$ ($0.1-5.1x10^7$)	$1.9x10^8$ ($0.01-4.4x10^8$)	$3.3x10^8$ ($0.02-8.6x10^8$)	$1.5x10^8$ ($0.04-5.1x10^8$)
CPC	$1.8x10^7$ ($0.02-1.0x10^8$)	$5.4x10^7$ ($0.0-1.7x10^8$)	$1.2x10^8$ ($0.0-6.1x10^8$)	$7.2x10^8$ ($0.03-2.9x10^9$)

Table 2. The ten most commonly isolated operational taxonomic unites (OTUs) from each library.

OTU	# Clones	% Total	Phylum (%Confidence)	Best Match in GenBank	Similarity
F1	204	6.0	Firmicutes (100%)	Tricochccus flocculiformis	97-98%
F2	151	4.4	Firmicutes (100%)	AY438851	98-99%
F3	142	4.2	Firmicutes (100%)	AF371787	99%
F4	96	2.8	Firmicutes (100%)	AY100573	98-99%
F5	86	2.5	Firmicutes (100%)	AY438899	98%
F6	83	2.4	Firmicutes (100%)	AY438880	97-98%
F7	77	2.3	Firmicutes (100%)	Clostridium lituseburense	98-100%
F8	60	1.8	Bacteroidetes (100%)	AY439932	98-99%
F9	58	1.7	Bacteroidetes (100%)	AB219992	92-94%
F10	58	1.7	Proteobacteria (100%)	Pseudomonas sp. SKU	98-99%
Aero1	154	9.6	Proteobacteria (100%)	Thauera terpenica	99-100%
Aero2	57	3.5	Actinobacteria (100%)	Aeromicrobium marinum	97-98%
Aero3	54	3.4	Proteobacteria (100%)	Pseudomonas sp. SKU	98-100%
Aero4	48	3.0	Proteobacteria (100%)	Dyella japonica	96-98%
Aero5	40	2.5	Proteobacteria (100%)	Roseobacter sp. YS-57	99%
Aero6	35	2.2	Bacteriodetes (100%)	Sphingobcterium thalpophilum	97-98%
Aero7	34	2.1	Proteobacteria (100%)	Xanthomonas axanopodis	95-97%
Aero8	34	2.1	Bacteriodetes (96%)	UBA3181A2	95-96%
Aero9	33	2.1	Proteobacteria (100%)	Dyella koreensis	97-98%
Aero10	26	1.6	Bacteriodetes (99%)	AF507866	96-97%
Anro1	144	8.6	Bacteriodetes (100%)	CR933150	97-99
Anro2	139	8.3	Firmicutes (100%)	Sedimentibacter sp. B4	96-97
Anro3	130	7.8	Firmicutes (88%)	DQ191708	96-97
Anro4	125	7.5	Firmicutes (100%)	Clostridium lituseburense	98-100
Anro5	58	3.5	Actinobacteria (62%)	AB092855	99-100
Anro6	43	2.7	Firmicutes (100%)	AY438851	98
Anro7	32	1.9	Proteobacteria (62%)	AB232563	96-97
Anro8	31	1.8	Firmictues (100%)	Trichococcus flocculiformis	97-98
Anro9	31	1.8	Firmicutes (100%)	Eubacterium tenue	99-100
Anro10	30	1.8	Firmicutes (100%)	AY100573	98-99
AS1	194	17.6	Proteobacteria (100%)	Thauera terpenica	99-100
AS2	58	5.3	Planctomycetes (100%)	Pirellula sp	98-99
AS3	31	2.8	Proteobacteria (100%)	Roseobacter sp. SY-5	97-99
AS4	20	1.8	Proteobacteria (100%)	Rhodobacter gluconicum	99-100
ANS5	19	1.7	Firmicutes (100%)	AY570630	98-99
AS6	18	1.6	Proteobacteria (100%)	Xanthomonas axonopodis	95-96
AS7	16	1.5	Firmicutes (100%)	Tissierella praeacuta	95-95
AS8	16	1.5	Proteobacteria (100%)	AY438740	99
AS9	16	1.5	Proteobacteria (100%)	Thermononas hydrothermalis	95-97
AS10	15	1.4	Bacteroidetes (53%)	Petrimonas sulfuriphila	98-99
ANS1	80	7.3	Firmicutes (100%)	Clostridium lituseburense	98-100
ANS2	64	5.9	Firmicutes (100%)	DQ191708	95-96
ANS3	53	4.9	Firmicutes (100%)	Turicibcter sanguinis	99-100
ANS4	48	4.4	Planctomycetes (100%)	Pirellula sp.	98-100
ANS5	47	4.3	Firmicutes (100%)	Sedimentibacter sp. B4	96-97
ANS6	46	4.2	Proteobacteria (100%)	Pseudomonas sp. SKU	99-100
ANS7	46	4.1	Bacteriodetes (100%)	AY953168	97-98
ANS8	33	3.0	Bacteriodetes (100%)	CR933150	98-99
ANS9	30	2.7	Firmicutes (100%)	Eubacterium tenue	99-100
ANS10	28	2.6	Actinobacteria (100%)	AB092855	99-100
CONT1	166	14.5	Firmicutes (100%)	Clostridium lituseburense	98-100
CONT2	48	4.2	Firmicutes (100%)	Turicibcter sanguinis	99-100
CONT3	36	3.2	Firmicutes (100%)	AF371787	99
CONT4	35	3.1	Firmicutes (100%)	Eubacterium tenue	99-100
CONT5	30	2.6	Spirochaetes (64%)	AY228699	97-98
CONT6	24	2.1	Bacteriodetes (100%)	AY439932	98-99
CONT7	20	1.8	Proteobacteria (100%)	Pseudomonas sp. SKU	99-100
CONT8	19	1.7	Firmicutes (100%)	AY622268	94-95
CONT9	19	1.7	Firmicutes (100%)	AY438851	98-99
CONT10	17	1.5	Bacteriodetes (100%)	AY953229	96-98

Figure 3. Dendogram of the Most Prevalent OTUs